

Mechanical Properties of the Isolated Catch Apparatus of the Sea Urchin Spine Joint: Muscle Fibers Do Not Contribute to Passive Stiffness Changes

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Abstract. The catch apparatus (CA) is the collagenous ligament at the spinal joint of sea urchins. It maintains spine posture by stiffening and allows spine movement by softening. A CA preparation, which was isolated from ossicles, was used to test the hypothesis that frictional forces between collagen fibers and ossicles are the source of stiffness changes. Isolated preparations of the CA changed in stiffness, thus falsifying the hypothesis. Another hypothesis proposes that muscle fibers, which represent a relatively small component of the CA, cause stiffening of the CA by contraction. Chemicals that evoked contraction in spine muscles did not always stiffen the CA: the CA of *Heterocentrotus mammillatus* softened in response to artificial seawater with potassium concentration elevated to 100 mM. This provided evidence against the muscle-based hypothesis. The present results suggest that the stiffness changes of the CA are based on changes in the mechanical properties of the extracellular components of the connective tissue and are therefore related to the connective tissue catch that is widespread in other echinoderms.

Introduction

Echinoderms have connective tissues that change their mechanical properties under neural control (Wilkie, 1996a). Connective tissue with such mutability is called catch connective tissue or mutable connective tissue, and the mechanical activity the tissue shows is referred to as connective tissue catch. The idea of connective tissue catch was put forward by Takahashi, based on studies on the spinal joint

ligament of sea urchins (Takahashi, 1967a, b). This ligament changes its stiffness, although it is made mostly of collagenous tissue. It stiffens to hold the spine in position and softens to permit spine movement under nervous control. Takahashi named this ligament the catch apparatus (CA), because in its long-lasting holding capacity it is similar to molluscan catch muscle. Since Takahashi's pioneering work, catch connective tissue has been found in various anatomical locations in all extant echinoderm classes (Wilkie, 1996a). It has been regarded as one of the major features that characterize the phylum Echinodermata (Motokawa, 1988; Ruppert and Barnes, 1994).

It is obvious that muscles are not involved in the stiffening mechanism of catch connective tissues containing no muscle cells at all (Birenheide *et al.*, 1996, 2000). It is sometimes the case, however, that a few muscle cells are found in catch connective tissue. The role of these muscles has been studied in detail only in the CA of sea urchins. The CA is made of collagen fibrils that are parallel to the long axis of spine, and thin myocytes that contain only several thick filaments are found among the fibrils (Smith *et al.*, 1981). Hidaka and Takahashi (1983) measured the tensile stiffness of the CA and the cross-sectional area of the muscles occupying it. If the muscles directly bear all the tensile forces of stiffened CA, the muscles would produce a greater force than that produced by the strongest muscles known elsewhere in the animal kingdom. The authors concluded that the muscles are not responsible for the stiffness changes.

A different interpretation of the role of these muscles was proposed by del Castillo *et al.* (1995). In the CA, bundles of collagen fibrils insert into the calcite stereom of the spinal ossicles and wind around the pillars of the stereom. These authors suggested that the frictional forces between collagen

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bundles and the pillars are responsible for the resistance to the stretch of the CA. When the CA is soft, a gap appears between the collagen bundles and pillars, which permits the bundles to slide freely. Contraction of the muscles, they proposed, presses the collagen bundles tightly against the pillars, which prevents the bundles from sliding and thus makes the CA inextensible and stiff. This hypothesis invited immediate debate (Wilkie, 1996b; del Castillo and Smith, 1996; Perez-Acevedo *et al.*, 1998). Both sides of the debate invoked Occam's razor. Del Castillo's hypothesis is applicable only to ligaments that contain muscles and are attached to skeletal elements. Wilkie criticized the hypothesis because it is more parsimonious to assume that all catch connective tissues share a common mechanism. Del Castillo regarded his hypothesis as being more parsimonious because the combination of muscles and ordinary connective tissue could account for the extraordinary holding capacity and eliminate the need to suppose that the collagenous ligaments have any unusual properties.

Another challenge to the widely accepted notion that all the catch connective tissues share a common non-muscular mechanism was put forward by Elphick and Melarange (2001). Drugs that cause stiffening of catch connective tissue, such as acetylcholine and the neuropeptide NGI-WY-amide, also induce contraction of muscles. This led these authors to suggest that muscle contraction is the common mechanism underlying the stiffening response of all the catch connective tissues. Wilkie (2002) again criticized this generalization by extensively reviewing the pharmacological works on catch connective tissues.

The aim of the present work was to study in detail catch connective tissue containing myocytes, using the CA as a model. The hypotheses of del Castillo and of Elphick and Melarange were examined by careful comparison of different methods and different species. These hypotheses are falsifiable. The hypothesis of del Castillo depends on the occurrence of friction between ossicles and collagen fibers. It predicts that stiffening will not be observed when the CA is isolated from the ossicles. The hypothesis of Elphick and Melarange predicts that every agent inducing muscle contraction should stiffen the CA. We used artificial seawater with elevated potassium concentration (KASW) as such an agent—elevated potassium being a universal muscle contractant that depolarizes the muscle cell membrane (Hodgkin and Horowitz, 1960). If KASW induces softening of the CA, the hypothesis is falsified.

Materials and Methods

Heterocentrotus mammillatus (Linnaeus), the slate-pencil sea urchin, was used mainly because of the large size of its catch apparatus (CA), which enabled us to isolate a block of connective tissue with precise dimensions. The sea urchins were collected near Sesoko Station, Tropical Biosphere

Research Center, University of the Ryukyus, and were kept in an aquarium of artificial seawater at 24–25 °C in our laboratory. The longer diameter at ambitus was 6–8 cm. Specimens of *Diadema setosum* (Leske) were collected near Misaki Marine Biological Station, University of Tokyo. They were kept in an aquarium of natural seawater at 18 °C. The diameter at ambitus of these animals was 6–7 cm.

Isolated catch apparatus

The sea urchin spine forms a ball-and-socket-like joint with a projection on the test called the tubercle. The spine base is connected to the tubercle by three layers of conical tissues that encapsulate the joint. The most superficial layer is a thin epidermis, under which there is a layer of spine muscle, and then the CA. In *Diadema setosum*, the center of both the spine base and the tubercle is perforated and houses a central ligament connecting the two ossicles. *Heterocentrotus mammillatus* lacks the central ligament.

The isolated CA was prepared by cutting the CA free from both the junction between it and the spine base and that between it and the tubercle. The procedure was as follows. A piece of a test with a spine was cut out. All the epidermis and spine muscles covering the CA were scraped off. In *H. mammillatus*, a block of the CA was isolated and trimmed. The size of the piece for mechanical tests was 4.0 mm in the direction of the spine axis, 2.0 mm in the circumferential direction of the joint, and 1.0 mm in the direction towards the center of the joint. The isolated CA piece of *D. setosum* was trimmed to the size 4.0 mm in the circumferential direction and 1.0 mm in the direction of spine axis. The direction towards the center was about 0.3 mm, which was the non-trimmed thickness of the CA.

The CA preparation with ossicles attached was prepared as follows. All the epidermis and spine muscles covering the CA were scraped off. Most of the CA was removed, leaving a strip of CA 2 mm wide in the circumferential direction connecting the spine and the tubercle.

Creep test

Mechanical properties of the CA were measured by creep tests. The CA was subjected to a tensile load, and the elongation of the CA was measured. The tension was in the direction of the axis of the spine except in the isolated CA of *D. setosum*, in which the tension was in the circumferential direction of the spinal joint. One end of the preparation was glued to the aluminum holder at the bottom of a trough. The opposite end was attached to a clip that was connected to a lever by a thread. The trough was filled with artificial seawater (ASW), and the sample was rested for 10–15 min. A load was applied to the sample *via* the lever. The load was 1.67–16.7 g for *H. mammillatus* and 0.67–3.33 g for *D. setosum*. The preparation elongated under the load. The elongation was measured with a laser displace-

ment sensor (3Z4M-J1001-6, Omron, Japan) and recorded with a data logger (NR-2000, Keyence, Japan). The rate of elongation became rather constant in 10–20 min, and the constant rate was kept for about 1 h (see Results). Chemical stimulation was applied during this phase of constant elongation. The normal viscosity was calculated as the stress divided by the strain rate (Motokawa, 1982). It was expressed as the relative value normalized by the value at the exact time when the chemical stimulus was applied. Here we call the normal viscosity simply "viscosity."

The composition of the ASW was NaCl, 433.7 mM; KCl, 10.0 mM; CaCl₂, 10.1 mM; MgCl₂, 52.5 mM; NaHCO₃, 2.5 mM (pH 8.1). Artificial seawater with its potassium concentration elevated to 100 mM (KASW) was prepared by reducing the sodium concentration so as to keep the osmotic concentration constant. Acetylcholine chloride was purchased from Nacalai Tesque, Japan. It was diluted in ASW to the final concentration of 10⁻⁶–10⁻³ M. In the control tests, ASW without chemicals was applied. All experiments were carried out at room temperature (22–28 °C). The temperature did not vary more than 2 °C in a single experiment.

Contraction of spine muscle

The isotonic contraction of the muscle bundles at the spinal joint was measured. A cut was made through the joint between the spine base and the mamelon, on which the spine base was mounted, leaving a strip of the spine muscle bundles connecting the spine base and the tubercle. A load was applied, and the shortening of the muscle bundle was measured at chemical stimulation, using the same set-up as for the creep tests. In *H. mammillatus* the width of the muscle bundle was 4 mm and the load was 0.67–1.67 g. In *D. setosum* the width was 2 mm and the load was 0.33–1.67 g.

Results

Isolated catch apparatus of *Heterocentrotus mammillatus*

The CA elongated rapidly on application of the load; in 10–20 min the elongation rate gradually decreased to a more-or-less constant value that was maintained for about 1 h (Fig. 1). Thereafter the rate increased rapidly until finally the CA ruptured.

Acetylcholine (ACh) at concentrations of 10⁻⁶–10⁻³ M decreased the creep rate of the isolated CA (Fig. 2a). The decrease was apparent in about 20 s after the application of ACh, and the rate reached its lowest value in about 3.5 min. The rate kept decreasing as long as ACh was applied. When ACh was washed out with pure artificial seawater (ASW), the rate increased again to its value before the application of ACh. ACh was effective in all the samples at 10⁻³ M, whereas at 10⁻⁶–10⁻⁴ M, 20%–30% of the samples failed

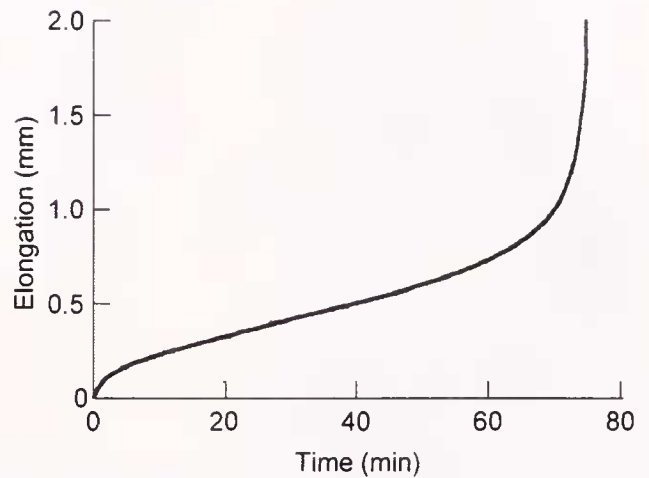


Figure 1. Typical creep curve of the isolated catch apparatus of *Heterocentrotus mammillatus*. It shows three phases: a fast elongating phase; a constant creep-rate phase; and a phase with increasing creep rate ending with tissue rupture. The sample broke at the right extremity of the curve.

to respond (Fig. 2b). The dose-response curve of the viscosity 2 min after the application of ACh is shown in Figure 2b. The viscosity in 10⁻³ M ACh was 2.88 ± 0.59 (average ± SEM, *n* = 10), which was significantly different (*P* < 0.01) from that of the control, as shown by the Mann-Whitney *U*-test.

Artificial seawater with elevated potassium (KASW) increased the creep rate in all samples of the isolated CA (Fig. 3a). The increase was apparent in about 20 s after the application of KASW and maximized in about 2 min. When KASW was washed out with ASW, the rate decreased again to the value before application of KASW. The viscosity 2.5 min after the application of KASW was 0.53 ± 0.09 (average ± SEM, *n* = 11), which was significantly different (*P* < 0.01) from that of the control.

Figure 3b shows the response of the CA with ossicles attached. KASW reversibly increased the creep rate as in the isolated CA. Such responses were found in 7 samples out of 9; there were no responses in 2 samples. The viscosity 2.5 min after the application of KASW was 0.77 ± 0.09 (average ± SEM, *n* = 9), which was not significantly different (*P* > 0.1) from the average of the isolated CA in KASW.

Isolated catch apparatus of *Diadema setosum*

Acetylcholine (ACh) at concentrations 10⁻⁶–10⁻³ M reversibly decreased the creep rate of the isolated CA (Fig. 4a). Although these preparations were loaded circumferentially, their response was quite similar to that of the isolated CA of *H. mammillatus*, which was loaded longitudinally. The dose-response curve of the viscosity 2 min after the application of ACh is shown in Figure 4b. The viscosity in 10⁻³ M ACh was 2.24 ± 0.71 (average ± SEM, *n* = 12),

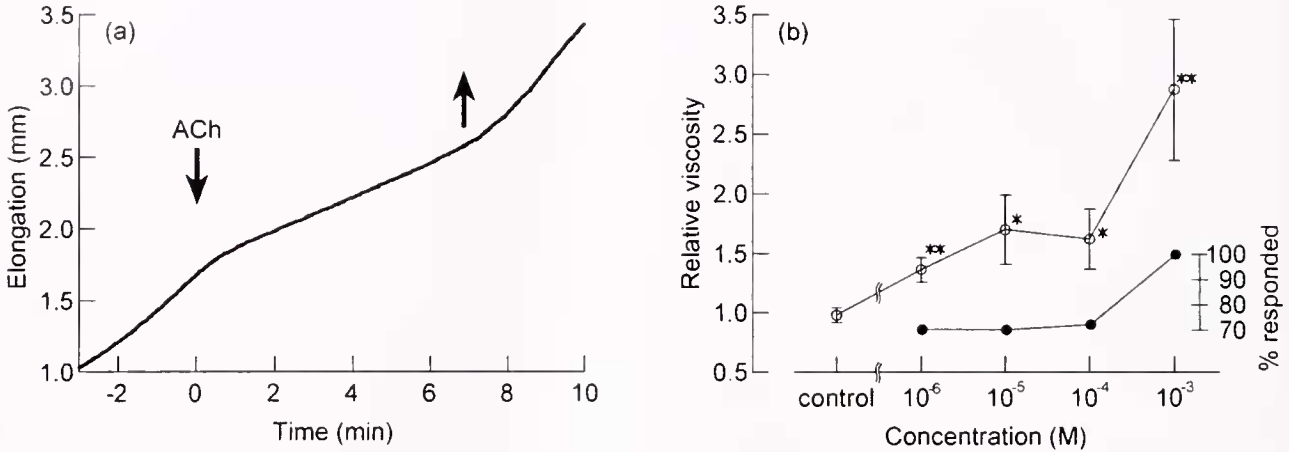


Figure 2. Response of the isolated catch apparatus of *Heterocentrotus mammillatus*. (a) Decrease in creep rate in response to 10^{-6} M ACh. In this and the following figures, downward arrows indicate the application of chemicals and upward ones indicate the time of washing. (b) Dose-response curves 2 min after the application of ACh. In the control test, pure ASW was applied. The average at every ACh concentration was statistically different from that of the control, as shown by the Mann-Whitney *U*-test (*, $P < 0.05$, **, $P < 0.01$). Error bar, \pm SEM. Filled circles denote percentage of the samples that responded.

which was significantly different ($P < 0.01$) from that of the control. ACh 10^{-6} – 10^{-3} M was effective in 70%–92% of samples tested (Fig. 4b).

In contrast to its effect on the CA of *H. mammillatus*, KASW decreased the creep rate of the isolated CA of *D. setosum* (Fig. 5). The decrease was apparent in about 20 s after the application of KASW, and the rate reached its lowest value in 2 min. When KASW was washed out by ASW, the rate increased again to the value before application of KASW. The viscosity 2.5 min after the application of KASW was 1.49 ± 0.11 (average \pm SEM, $n = 30$), which was significantly different ($P < 0.01$) from that of the controls. The response to KASW was seen in 19 samples out of 30; there were no changes in the elongation rate in the other 11 samples.

Contraction of spine muscles

The spine muscles of *H. mammillatus* and *D. setosum* contracted in response to ACh and to KASW. The responses to ACh were similar in the two species. The contraction in low concentrations of ACh (10^{-6} – 10^{-5} M) was phasic; the shortening reached a peak in about 1 min and was soon followed by rapid relaxation. Complete relaxation was observed in 3–4 min, even in the presence of ACh (Fig. 6a, d). In higher concentrations of ACh (10^{-4} – 10^{-3} M), a peak was followed by oscillations that lasted as long as ACh was present (Fig. 6b, e). Similar results have been reported for the spine muscle of *Anthocidaris crassispina* (Shingyoji and Yamaguchi, 1995). KASW caused a tonic contraction in *H. mammillatus* (Fig. 6c). In *D. setosum*, KASW caused a

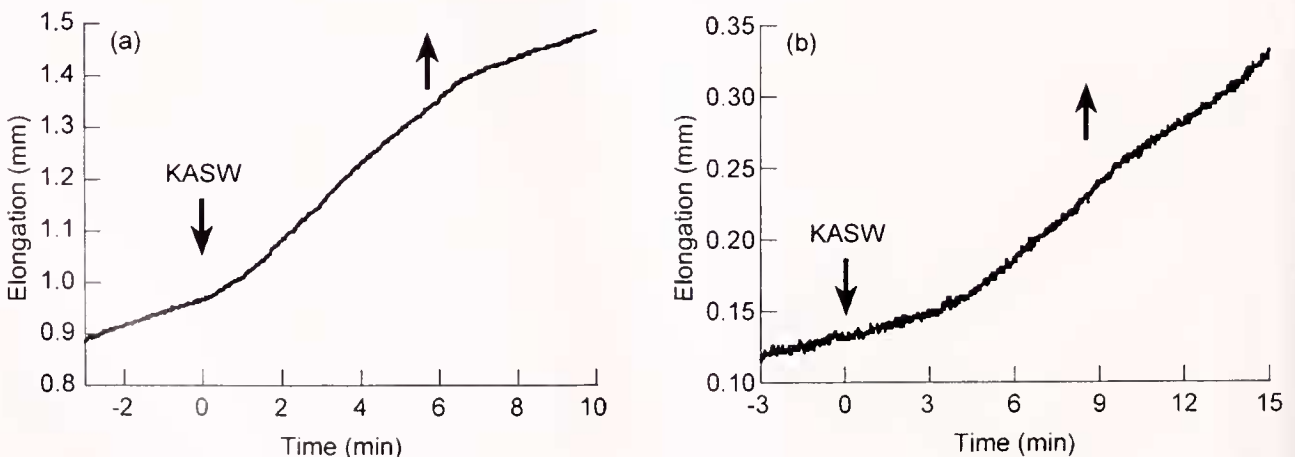


Figure 3. Increase in creep rate of catch apparatus of *Heterocentrotus mammillatus* in response to artificial seawater with elevated K concentration (KASW): (a) without ossicles; (b) with ossicles attached.

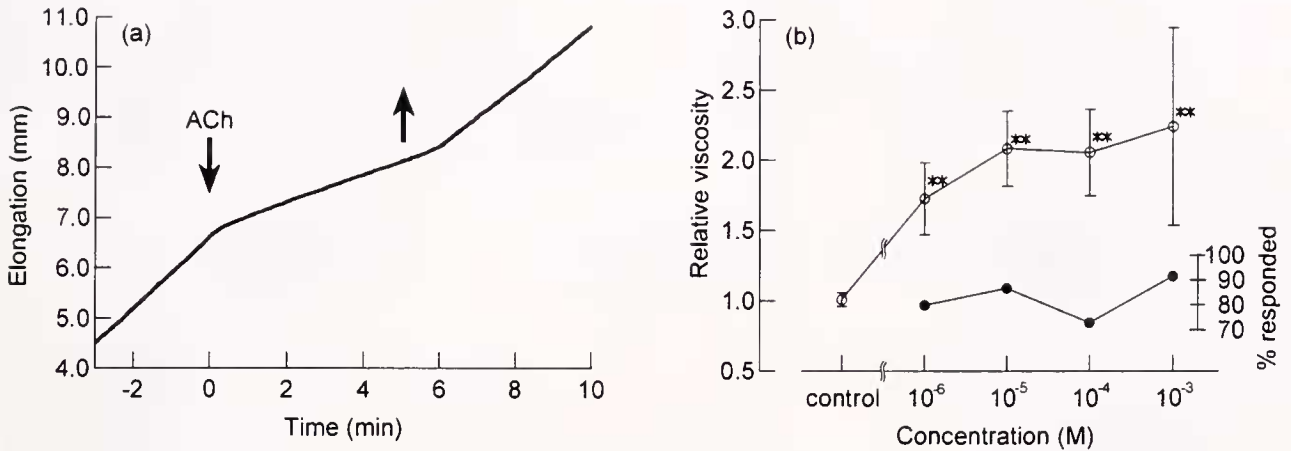


Figure 4. Response of the isolated catch apparatus of *Diadema setosum*. (a) Decrease in creep rate in response to 10^{-6} M ACh. (b) Dose-response curves. Hollow circles give the viscosity 2 min after the application of ACh. The average at every ACh concentration was significantly different from the control, as shown by the Mann-Whitney *U*-test (**, $P < 0.01$). Error bar, \pm SEM. Filled circles denote percentage of the samples that responded.

contraction with two peaks (Fig. 6f); the contraction after the second peak decreased little or slowly.

Discussion

The mechanical properties of the isolated catch apparatus (CA) and their response to stimulation were studied for the first time. The creep curve of the isolated CA had three phases, quite similar to those of the CA with ossicles in *Anthocidaris crassispina* (Takahashi, 1967b). The responses to acetylcholine (ACh) were also similar to those of the attached CA. ACh decreases the creep rate of the attached CA of *A. crassispina* (Takahashi, 1967b) and *Heterocentrotus mammillatus* (Motokawa, 1981). We showed

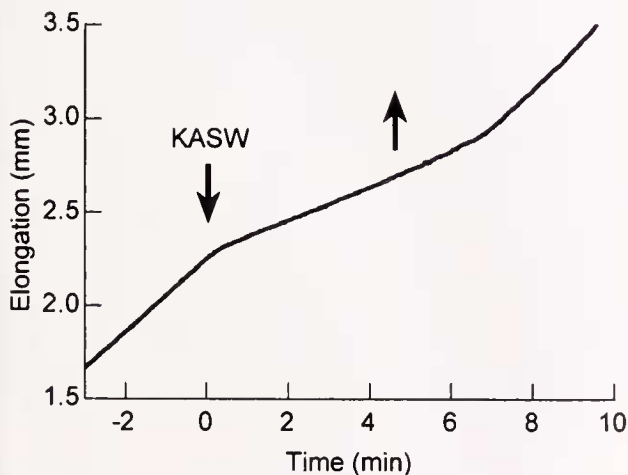


Figure 5. Increase in creep rate of catch apparatus of *Diadema setosum* in response to artificial seawater with elevated K concentration (KASW).

that the CA of both *H. mammillatus* and *D. setosum*, from which ossicles had been removed, also undergoes a decrease in creep rate in response to ACh. Artificial seawater with elevated potassium (KASW) increased the creep rate of the CA of *H. mammillatus* irrespective of whether ossicles were attached or not. The extent of the changes in the viscosity of the isolated CA was not different from that of the attached CA. All samples of the isolated CA of *H. mammillatus* responded to 10^{-3} M ACh and to KASW. The percentage of the samples that responded was higher than that of the attached CA. Thus the responses were qualitatively and quantitatively the same or even better in the isolated CA. These results suggest strongly that the mechanical properties and the responsiveness to stimuli of the CA are not impaired by isolation from the ossicles.

The isolated CA either increased or decreased its elongation rate in response to stimuli. Hereafter, we refer to a decrease in the elongation rate as stiffening and an increase as softening. The present results show clearly that the ossicles are not necessary for stiffness changes in the CA; thus they falsify the hypothesis of del Castillo *et al.* (1995), which proposed that frictional forces within the ossicles provide the mechanism of stiffness changes.

Our present study provided further evidence against the myocyte-based hypotheses. The CA of *H. mammillatus* softened in response to KASW. Media with an elevated potassium concentration generally cause contraction of muscles. The present study confirmed this in the spine muscles of *H. mammillatus* and *D. setosum*. Therefore, we would expect that the myocytes in the CA of *H. mammillatus* also contract in response to KASW. However, the CA of this species was softened by KASW. This suggests that the contraction of the myocytes in the CA has little effect on

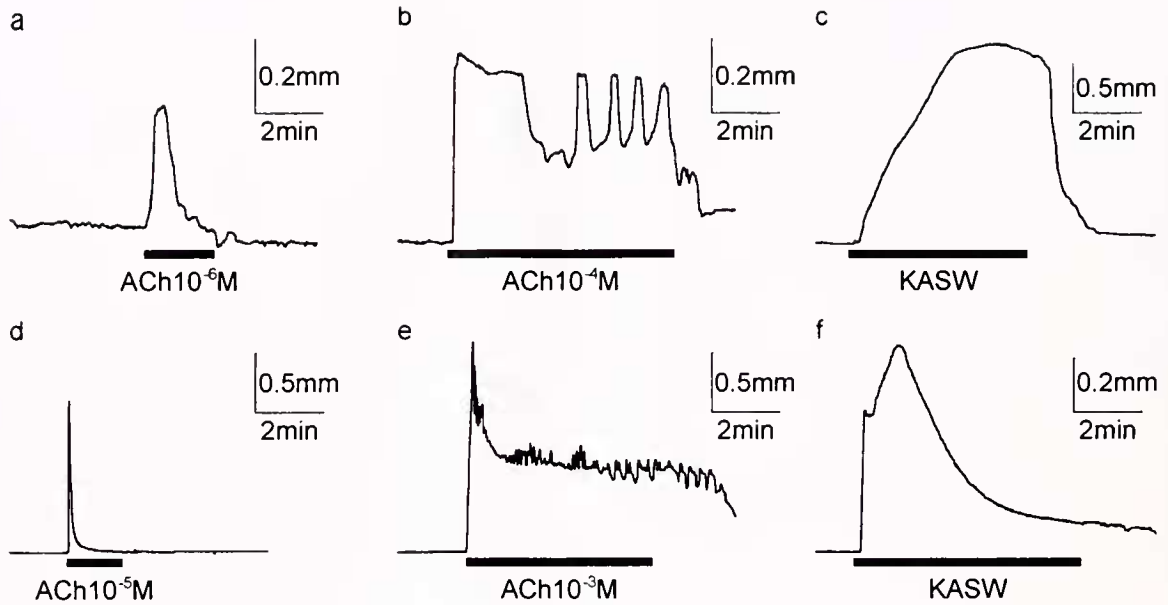


Figure 6. Contraction of spine muscles of *Heterocentrotus mammillatus* (a–c) and *Diadema setosum* (d–f). Heavy horizontal bars denote the application of chemical stimuli, either ACh or artificial seawater with elevated K concentration (KASW).

catch activities and thus provides the evidence against the myocyte-induced friction hypothesis of del Castillo *et al.* (1995) and against the hypothesis of Elphick and Melarange (2001), who claimed that the stiffening of all the catch connective tissues could be caused by muscles in the tissues.

However, the possibility remains that contraction of the myocytes in the CA may induce stiffening without the help of ossicles. The collagen bundles in the CA are parallel to the long axis of the spine. It is feasible that contraction of the myocytes could reduce the distance between adjacent bundles, thus permitting the formation of some temporary bonds between bundles. Once some bonds had been introduced, they would increase the resistance to stretch and thus stiffen the CA. Our creep experiments with the isolated CA of *D. setosum* provided evidence against this possibility. In this preparation the CA was stretched in the direction perpendicular to that of the axis of collagen bundles. Stiffening was observed even when this preparation was stretched to twice the original length. It would be highly unlikely that muscle cells oriented perpendicularly to the normal direction of the stretch could have been responsible for the stiffening of this preparation in which the distance between collagen bundles was extraordinarily large.

The time course of the muscular contraction also suggested that the sustained contraction of the myocytes is unlikely to be the basis of the catch mechanism. The CA remained stiff as long as 10^{-6} M ACh was applied for at least 7.5 min, while contraction of spine muscles induced by lower concentrations of ACh (10^{-6} M– 10^{-5} M) were phasic, with the contraction lasting for only 2 min.

The spinal articulation of *D. setosum* is provided with a central ligament which, in addition to the CA, connects the spine base to the tubercle. The central ligament contains no myocytes and yet shows stiffening to ACh and to KASW, as does the CA of this species (Motokawa, 1983). The structure of the central ligament resembles that of the CA, except that it lacks myocytes. It is thus parsimonious to suppose that similar ligaments found side by side employ the same catch mechanism that does not depend on myocytes. The central ligament is subjected to far less strain than is the CA at the same inclination angle of the spine. As the role of the myocytes in the CA may be to reshorten it after it has been stretched by spine movements, the lack of myocytes in the central ligament may be related to the reduced strain it experiences and the consequently reduced need for a special device to restore its original length after stretching.

The present study strongly suggests that the effector cells responsible for the variable stiffness of the CA are not myocytes. Although we do not fully understand the nature of the effector cells and the force-bearing components of catch connective tissues, all available evidence supports the general conclusions that the effector cells are some type of secretory cells and that the force-bearing components are extracellular materials, not muscle cells (Wilkie, 1996a). The differences in nerves controlling the secretory cells may account for the differences in the response to KASW.

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